1: J Mol Biol 1997 Oct 31;273(3):614-22

A conformational transition at the N terminus of the prion protein features in formation of the scrapie isoform.

Peretz D, Williamson RA, Matsunaga Y, Serban H, Pinilla C, Bastidas RB, Rozenshteyn R, James TL, Houghten RA, Cohen FE, Prusiner SB, Burton DR.

Department of Neurology, School of Pharmacy, University of California, San Francisco, CA 94143, USA.

The scrapie prion protein (PrPSc) is formed from the cellular isoform (PrPC) by a post-translational process that involves a profound conformational change. Linear epitopes for recombinant antibody Fab fragments (Fabs) on PrPC and on the protease-resistant core of PrPSc, designated PrP 27-30, were identified using ELISA and immunoprecipitation. An epitope region at the C terminus was accessible in both PrPC and PrP 27-30; in contrast, epitopes towards the N-terminal region (residues 90 to 120) were accessible in PrPC but largely cryptic in PrP 27-30. Denaturation of PrP 27-30 exposed the epitopes of the N-terminal domain. We argue from our findings that the major conformational change underlying PrPSc formation occurs within the N-terminal segment of PrP 27-30.

PMID: 9356250 [PubMed - indexed for MEDLINE]

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L9: Entry 3 of 4 File: USPT

DOCUMENT-IDENTIFIER: US 5962669 A
TITLE: Nucleic acid encoding prion protein variant

Detailed Description Text (253):
MoPrP codon 216 which encodes a Gln residue in both Mo and Hu PrP was mutagenized because the side chain of this residue protrudes from the opposite face of the C-terminal .alpha.-helix at positions 214 and 218 and a mutation causes inherited prion disease. A Swedish family with GSS has been reported with a Q.fwdarw.R mutation, Hu codon 217 corresponds to Mo codon 216. Although brain sections showed PrP amyloid plaques, extracts showed neither infectivity nor protease resistant PrP.sup.Sc on Western blots.

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L10: Entry 1 of 4 File: USPT

US-PAT-NO: 6365359
DOCUMENT-IDENTIFIER: US 6365359 B1

TITLE: Inhibitors of prion formation

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Prusiner; Stanley B.	San Francisco	CA		
Cohen; Fred E.	San Francisco	CA		
James; Thomas L.	Nicasio	CA		
Kaneko; Kiyotoshi	Kodaira			JP

US-CL-CURRENT: 435/7.1; 514/2, 530/350, 702/19

CLAIMS:

What is claimed is:

1. An assay to identify a PrP pharmacophore, said assay comprising the steps of:

determining functional residues of the PrP protein involved in prion complex interactions;

developing a plurality of three dimensional structures based on these functional residues;

comparing the plurality of three dimensional structures with a series of compounds having calculatable tertiary structures; and

identifying compounds having a spatial orientation consistent with binding PrP at the determined functional residues.

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L10: Entry 1 of 4 File: USPT Apr 2, 2002

US-PAT-NO: 6365359
DOCUMENT-IDENTIFIER: US 6365359 B1

TITLE: Inhibitors of prion formation

DATE-ISSUED: April 2, 2002

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Prusiner; Stanley B.	San Francisco	CA		
Cohen; Fred E.	San Francisco	CA		
James; Thomas L.	Nicasio	CA		
Kaneko; Kiyotoshi	Kodaira			JP

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The Regents of the University of California	Oakland	CA			02

APPL-NO: 09/ 439921 [PALM]
DATE FILED: November 12, 1999

PARENT-CASE:
CROSS-REFERENCE This application is a continuation-in-part application of Ser. No. 08/868,162, filed Jun. 2, 1997 now U.S. Pat. No. 5,962,669, and Ser. No. 09/076,606, filed May 12, 1998, each of which is incorporated herein by reference in their entirety and to which applications we claim priority under 35 USC .sctn.120.

INT-CL: [07] G01 N 33/53, C07 K 14/00

US-CL-ISSUED: 435/7.1; 574/2, 530/350
US-CL-CURRENT: 435/7.1; 514/2, 530/350, 702/19

FIELD-OF-SEARCH: 530/350, 435/69.1, 435/252.3, 435/320.1, 574/2

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5025388	June 1991	Cramer, III et al.	
☐	5307287	April 1994	Cramer, III et al.	
☐	5434796	July 1995	Weininger	
☐	5526281	June 1996	Chapman et al.	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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WO 93/10227	May 1995	WO	

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ART-UNIT: 1653

PRIMARY-EXAMINER: Carlson; Karen Cochrane

ABSTRACT:

Molecules are disclosed that interact with the cellular components involved in conversion of PrP.sup.C to PrP.sup.Sc. The molecules disclosed can be small molecules, peptides or protein analogs, e.g. analogs of PrP.sup.C. In one embodiment, these molecules interfere with prion formation and/or replication, e.g. by preventing interactions of proteins involved in a prion complex or by interfering with .beta.-sheet formation. In another embodiment, the molecules of the invention promote PrP.sup.C conversion to PrP.sup.Sc, e.g. by binding to PrP.sup.C and facilitating a conformational change from PrP.sup.C to PrP.sup.Sc.

1 Claims, 9 Drawing figures

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L10: Entry 3 of 4 File: USPT

DOCUMENT-IDENTIFIER: US 5750361 A
TITLE: Formation and use of prion protein (PRP) complexes

Detailed Description Text (46):
The method of the invention is optimized for transgenes for bioassay of human, bovine, ovine, and porcine prions. This approach has important implications for construction of a universal transgene for bioassay of human prions. As recently described in work from this laboratory, amino acid mismatches at residues 102 and 129, but not 200, result in substantial prolongation of the incubation time required for onset of PrP-mediated disease (Telling et al. (1995) supra). Identifying amino acid side chains at these and other critical residues that will permit transmission of all human prions without regard to the amino acid sequence of PrP.sup.Sc may be accomplished with the above described assay methodology. In addition to developing a universal transgene with respect to the sequence of PrP.sup.Sc, the above described approach may be utilized to create an artificial PrP.sup.C which interacts with human PrP.sup.Sc more efficiently than naturally occurring human PrP.sup.C itself. Thus, a more sensitive and rapid bioassay for human prions may be developed with the use of the invention.

L42: Entry 13 of 23 File: USPT

DOCUMENT-IDENTIFIER: US 5750361 A
TITLE: Formation and use of prion protein (PRP) complexes

YEAR ISSUED (ORACLE) (1):
1998

Brief Summary Text (28):
In another aspect, the invention features an assay for PrP.sup.Sc. The presence of PrP.sup.Sc in a sample is determined from the displacement of a PrP peptide from the prion protein complex. In one embodiment, a prion protein complex is allowed to form between a labelled PrP peptide and PrP.sup.C, and a test sample added. The amount of displaced peptide is determined by measuring the amount of label in the supernatant fraction after centrifugation to pellet the insoluble prion protein complexes. In another embodiment, the first component PrP.sup.C is affinity labeled and immobilized after prion protein complex formation with a second component labelled PrP peptide. A test sample is added, and the amount of displaced labelled peptide is determined in the unbound phase.

Detailed Description Text (36):
In one aspect, the invention features an assay for PrP.sup.Sc. Since PrP.sup.Sc binds tightly to PrP.sup.C, the displacement of labelled PrP peptide from the prion protein complex can be used to assay for the presence of PrP.sup.Sc. In one embodiment of this method of the invention, the displacement of labelled recombinant PrP (Hu 90-231) (SEQ ID NO:10) from the prion protein complex formed between PrP.sup.C and Hu 90-231 is used to assay for the presence of PrP.sup.Sc. In the PrP.sup.Sc assay method of the invention, either the PrP peptide or PrP component may be labelled. Appropriate labels are known to the art, and include radioisotopes, fluorescent dyes, or spectrophotometrically-detectable chromophores.

CLAIMS:

21. The method of claim 20, wherein the second component PrP peptide is labelled and the amount of PrP peptide displaced from the prion protein complex is measured by removing the PrP.sup.C /PrP.sup.Sc complex and determining the amount of label remaining.

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L44: Entry 13 of 35 File: USPT

DOCUMENT-IDENTIFIER: US 5837473 A
TITLE: Methods of screening for agents affecting the deposition of .beta.-amyloid peptides on amyloid plaques in human tissue

YEAR ISSUED (ORACLE) (1):
1998

Abstract Text (1):
The present invention provides a labelled .beta.-amyloid peptide or a labelled active fragment of .beta.-amyloid peptide and methods of using the labelled peptides to screen for agents which affect the deposition of the labelled peptide onto amyloid plaques in tissue samples evidencing the presence of Alzheimer's amyloidosis.

Brief Summary Text (8):
The present invention provides a labelled .beta.-amyloid peptide useful for detecting Alzheimer's disease and studying Alzheimer's disease-related conditions. A method for obtaining the labelled .beta.-amyloid peptide is also provided. The method employs essentially dry .beta.-amyloid peptide and rapid formation of a labelled .beta.-amyloid peptide. Preferably, the peptide has the amino acid sequence: H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-OH [SEQ. ID NO:1] or an active fragment of such amyloid peptide. In the preferred embodiment, the .beta.-amyloid peptide is bound to a radioactive label such as radioactive iodine. However, other appropriate labelling agents and techniques, for example, enzymatic or fluorescent labelling of the .beta.-amyloid peptide or active peptide fragment, can be used, either alone or in combination. The labelled peptide can be combined with a pharmaceutically acceptable carrier for in vivo diagnostic and possible therapeutic use.

Brief Summary Text (9):
The present invention relates to various uses of the labelled .beta.-amyloid peptide. One such use is in vitro detection and monitoring of Alzheimer's disease in a patient. This is accomplished by combining a sample of patient tissue with an amount of labelled .beta.-amyloid peptide or active fragment thereof for a period of time effective to allow binding of the labelled peptide or peptide fragment to the tissue. The bound labelled peptide/tissue complex is then detected and, if desired, quantified. In vitro detection and monitoring can be accomplished by numerous techniques, including autoradiographic or homogenate binding assays. Homogenate binding assays can be used to screen for potential therapeutic agents, in particular, the ability of these agents to affect deposition of .beta.-amyloid peptide onto tissue and existing plaques. This includes agents that inhibit or enhance deposition or are capable of breaking up existing plaques. Further, progression of Alzheimer's disease may be monitored by assaying a later-acquired sample of tissue from a patient earlier tested in the same manner as the earlier-acquired sample. The amount of bound peptide or peptide fragment in the two tissue samples is compared to provide an assessment as to the development of the disease in a patient.

Brief Summary Text (10):
According to the present invention, in vivo detection of Alzheimer's disease in a patient is also possible by administering the labelled .beta.-amyloid peptide or

active fragment to the patient and detecting the presence of the labelled peptide or peptide fragment bound to the tissue in the patient by known imaging techniques such as positron emission tomography (PET) imaging.

Brief Summary Text (12):
The labelled .beta.-amyloid peptide of the present invention and methods of use described herein provide qualitative and quantitative diagnostic tools for studying and potentially treating Alzheimer's disease. Use of .beta.-amyloid peptide aggregation as a screening tool for compositions having potential therapeutic use provides a previously unavailable technique to study and evaluate potential therapeutic agents without patient tissue.

Detailed Description Text (2):
The present invention provides: a labelled .beta.-amyloid peptide or active (i.e., biologically, or chemically active, or shown as positive in an assay) fragment; a composition including the labelled .beta.-amyloid peptide or active fragment thereof and a pharmaceutical carrier; a method for labelling the .beta.-amyloid peptide or an active fragment thereof, and methods of using the labelled peptide or peptide fragment for detecting or monitoring Alzheimer's disease in a patient.

Detailed Description Text (4):
Labelled .beta.-amyloid peptide or active fragments are used in the methods according to the invention. .beta.-amyloid peptide has a sequence of about 40 amino acids. The exact length of the naturally-occurring peptide may vary from about 39 to 43 amino acids, depending on the presence of ragged ends. The sequence of the 42-mer peptide is H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-OH [SEQ. ID NO:2], and the sequence of the 40-mer peptide is H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-OH [SEQ. ID NO:1]. The 40-mer peptide is preferred in the present invention. However, active fragments having as few as about 5 amino acids and ranging from about 5 to about 43 amino acid units are useful if appropriate labelling and measuring techniques are used to detect a smaller fragment of the 39- to 43-mer peptide. In particular, a peptide fragment derived from the 1-43 amino acid region of .beta.-amyloid peptide and having at least 10 amino acid units, as for example, a fragment containing the amino acids at about position 25-35, may be used according to the invention.

Detailed Description Text (6):
The .beta.-amyloid peptide or active fragment is combined with an acceptable label as described herein. The label can be radioactive, enzymatic, or fluorescent, or any combination thereof. Preferably, a radioactive label such as radioactive iodine-125 is used.

Detailed Description Text (14):
In vitro methods of detecting Alzheimer's disease according to the present invention combine an amount of sample of tissue obtained from a patient with an amount of labelled .beta.-amyloid peptide or active fragment thereof. The tissue sample may be obtained from any tissue in which the growth of amyloid plaques may occur, including, for example, the nasal epithelium, skin and tissue obtained from portions of the brain such as the cerebral cortex, hippocampus and amygdala, and the like. Preferably the tissue sample used is about 1-20 ug/assay tube for tissue prepared in thin section which is preferably about 5-15 .mu.m thick, and about 5-50 mg/assay tube for tissue prepared as a homogenate.

Detailed Description Text (15):
Submicrogram amounts of the labelled .beta.-amyloid peptide or active fragment thereof, for example, about 0.1 to 10 ng of .sup.125 I radiolabelled .beta.-amyloid peptide or fragment thereof, is added to each tissue sample for a time effective for the labelled peptide or peptide fragment to bind with the tissue sample. Preferably, the binding reaction time is about 1 to 5 hours, more preferably about 2 hours under the experimental conditions described herein. The time will vary depending on the specific experimental conditions, as will be understood by one skilled in the art. After reaction of the tissue sample with the labelled peptide or fragment, the tissue sample is preferably washed with an appropriate buffer to remove unbound labelled peptide. Homogenized tissue samples are preferably filtered prior to the washing step.

Detailed Description Text (19):
The in vitro detecting and monitoring techniques according to the present invention can be qualitative or quantitative. The presence of tissue-bound labelled peptide or peptide fragment may be detected according to known techniques appropriate for the particular labelling agent and method used (e.g., radioisotope, fluorophore, enzyme, antigen), the tissue sample type (e.g., homogenate, thin slice), the particular peptide or fragment used (e.g., .beta.-amyloid peptide.sup.1-40, .beta.-amyloid peptide.sup.25-35), and other factors of the assay. In addition, the method of detecting radioactive isotopes will vary according to the isotope and its corresponding energy level. For example, a gamma counter is capable of detecting .sup.125 iodine, but not tritium (.sup.3 H) or .sup.35 sulfur.

Detailed Description Text (22):
In vivo detection and monitoring of Alzheimer's disease includes administering the labelled .beta.-amyloid peptide or active fragment thereof to a patient in an amount effective to bind with tissue evidencing the presence of, or susceptible to, Alzheimer's disease. Like in vitro detecting methods, the presence of the labelled peptide or peptide fragment bound to tissue in the patient is detected by a known detecting technique that is appropriate to the tissue sample type, the particular peptide or fragment used, the labelling method used, and other such factors unique to the particular assay being performed.

Detailed Description Text (24):
The present invention also provides useful methods to detect, monitor and screen potential therapeutic agents for affecting Alzheimer's disease. In particular, methods for in vitro screening of agents that are capable of inhibiting or enhancing the aggregation of .beta.-amyloid peptide or active fragments thereof, including the ability to break up and, in certain cases, to inhibit formation or growth of plaques, are provided. According to one method, potential therapeutic agents are placed in competition with labelled .beta.-amyloid peptide in a solution with sample patient tissue, and the effect of the test agent on .beta.-amyloid peptide binding to same tissue is quantified.

Detailed Description Text (28):
In yet another embodiment, the invention provides a pharmaceutical composition for in vivo use in detecting Alzheimer's disease in a human tissue. The composition contains labelled .beta.-amyloid peptide or active peptide fragment thereof, in a pharmaceutically-acceptable carrier of the type appreciated by those of skill in the art. The composition contains the labelled peptide or peptide fragment in an amount effective to bind to tissue evidencing the presence of, or susceptible to, Alzheimer's disease, when administered in vivo.

Detailed Description Text (29):
The labelled .beta.-amyloid peptide or peptide fragment is useful to detect or quantify the presence of, or tissue susceptibility to, Alzheimer's disease in human tissue. With respect to an in vitro tissue binding assay, the amount of labelled peptide or fragment is effective to bind with tissue evidencing the presence of, or susceptibility to, Alzheimer's disease. Such a binding assay can be used to test agents that may be useful anti-Alzheimer's disease compositions.

Detailed Description Text (32):
Preparation of Labelled .beta.-Amyloid Peptide

Detailed Description Text (33):
A radiolabelled-amyloid-peptide, .sup.125 I-labeled .beta.-amyloid peptide.sup.1-40, was synthesized for use in determining binding properties of human .beta.-amyloid peptide tissues in homogenates, and to characterize binding to localize tissue sites with which the peptide interacts in thin sections of normal or Alzheimer's disease tissue including central nervous system and vascular tissue.

Detailed Description Text (45):
It was determined that in order to avoid aggregation of the labeled peptide and consequent loss of viable tracer during the reduction reaction, the reaction time must be 90 minutes or less. Although reduction of the sulfoxide form is not complete

at 90 minutes, the labelled .beta.-amyloid peptide remains mostly as intact monomer. Longer reaction times provide more complete reduction at the risk of formation of unusable aggregates.

Detailed Description Text (51):
Detection of In Vivo .beta.-Amyloid Peptide Deposition in Human Tissue and Use of Labelled .beta.-Amyloid Peptide

Detailed Description Text (55):
In Alzheimer's disease tissue homogenates, about 20,000 cpm (20%) of the .sup.125 I-labeled .beta.-amyloid peptide.sup.1-40 was bound in the absence of unlabelled peptides, and 10000 cpm (10%) in the presence of 10.sup.-5 M unlabelled .beta.-amyloid peptide.sup.1-40. No displacement binding was observed when plaques were absent (i.e., in normal tissue). Autoradiography of the homogenate confirmed that the highest density of binding sites in Alzheimer's disease tissue was on intact plaques.

Detailed Description Text (61):
Visualization of the binding sites for .sup.125 I-.beta.-amyloid peptide.sup.1-40 by autoradiography showed that the ligand was deposited on amyloid plaques at both parenchymal and vascular sites in Alzheimer's disease brain. Thus, in the Alzheimer's disease cerebral cortex, .sup.125 I-.beta.-amyloid peptide.sup.1-40 was deposited on both the core and the halo of essentially every extracellular plaque examined (FIG. 2). In the Alzheimer's disease cerebellar cortex, diffuse plaques which were not visualized with thioflavin S were readily labelled with the .beta.-amyloid peptide radioligand (see FIG. 2), and clear morphological differences between these plaques and the compact plaques of the cerebral cortex were evident. Thus, both the classic senile plaques of the cortex and the diffuse nonneuritic deposits of the cerebellum were found capable of in vitro growth by addition of .beta.-amyloid peptide from dilute solution. While thioflavin S and anti-A4 antibodies stained neurons outside the plaques as well as the plaques themselves, deposition of the radioligand was limited to the plaques alone (see FIG. 2). Furthermore, the sensitivity of detection of plaques with the radioligand far exceeded that of dyes or antibodies. Thus, the radioligand was capable of detecting more lesions at a potentially earlier time than detection techniques reported to date. In cerebral cortex tissue obtained from normal brain, there was essentially no deposition of .beta.-amyloid peptide radioligand detected above background levels at parenchymal or vascular sites.

Detailed Description Text (62):
Vascular plaques were visualized by .beta.-amyloid peptide deposition in approximately 0-20% of intra- and extraparenchymal blood vessels in Alzheimer's disease brain, although the fraction of vessels labelled showed considerable variation between cortical areas examined and between patients (See Table II). The cerebrovascular deposits were consistently labelled more densely than cerebral plaques within the same section. In vessels seen in transverse section (see FIG. 3), the deposition of .beta.-amyloid peptide radioligand was not uniform but concentrated in a part of the vessel, apparently the tunica media. Endothelial tissue was not labeled.

Detailed Description Text (64):
The mammalian tachykinins, substance P and neurokinins A and B, over a broad range of concentration, did not inhibit the deposition of radiolabelled .beta.-amyloid peptide onto Alzheimer's disease plaques (see FIG. 1). Binding sites for radiolabelled tachykinins were present in both Alzheimer's disease and normal tissue, and were not associated with plaques. These tachykinin binding sites were indistinguishable from the tachykinin receptors that have been described by J. E. Maggio, Ann. Rev. Neurosc., 11:13 (1988), and P. W. Nantyh et al., Proc. Natl. Acad. Sci., 86:5193 (1989), with unlabelled tachykinins displacing their radiolabelled analogues at nanomolar concentrations. In contrast, there was no displacement of any of the tachykinin radioligands by .beta.-amyloid peptide.sup.1-40 at concentrations up to 30 .mu.M, which indicates that the amyloid peptide does not interact with tachykinin receptors under the standard conditions labelling tachykinin receptors as described. These results were consistent with the reported structure-activity studies among the tachykinin peptide family, namely, that a carboxyl-terminal amide

is required for activity.

Detailed Description Text (70):
The experiments described in Example 1 demonstrated that amyloid plaques can grow in vitro by deposition of labeled amyloid peptide from dilute solution. The following experiment with radiolabelled .beta.-amyloid peptide demonstrated that the peptide can aggregate in vitro in the absence of amyloid plaques. This latter property provides an in vitro system for qualitative and quantitative evaluation of agents or conditions that may inhibit or enhance this aggregation or disperse preformed aggregates. Agents so identified may have similar effects on Alzheimer's disease plaques in vivo.

Detailed Description Text (71):
A solution of about 10.sup.-9 M labelled .beta.-amyloid peptide in 50 mM Tricine buffer at pH 7.5 was prepared from stock solution of the peptide as described hereinabove in Example 1. The solution was aliquotted into several reaction vessels (polypropylene microcentrifuge tubes) and allowed to stand at room temperature with occasional vortex mixing. At various times, the tubes were centrifuged at 12000g for 4 minutes, and the fraction of initial (t=0) cpm of labelled peptide remaining in the supernatant fraction determined by removing a small aliquot for counting. The time course of the disappearance of the tracer from the supernatant under these conditions is shown in FIG. 4. The rate of disappearance was dependent on a variety of other conditions which were evaluated using this assay. Thus, the rate of the disappearance depends on peptide concentration (faster at higher peptide concentrations), and on ionic strength (faster at higher salt concentrations). The rate of disappearance was further dependent on the presence of certain detergents such as sodium dodecylsulfate (SDS) (faster in 0.01% SDS; see, FIG. 4), and on the presence of certain organic solvents (slower in the presence of acetonitrile).

Detailed Description Text (72):
Methods similar to those described hereinabove may be used to assay the rate of formation of aggregates which may be separated by filtration or centrifugation, or the rate of dispersion of aggregates of amyloid peptide, or the effects of various agents on these processes. In each case, a key step is the use of labelled amyloid peptide in the aggregate or in solution to follow the time course of the process.

Detailed Description Text (84):
A dark field photomicrograph showing the distribution of .sup.125 I-.beta.-amyloid peptide.sup.1-40 in Alzheimer's disease temporal cortex revealed numerous plaques throughout the grey matter (FIG. 2(a)). A dark-field micrograph of a serially adjacent section as treated in FIG. 2(a), except that 10.sup.-5 M cold .beta..sub.1-40 was added to the incubation medium, is shown in FIG. 2(b). An immunohistochemistry of amyloid deposits using antibodies raised against .beta.-amyloid peptide.sup.1-40 (amyloid peptide A4) in Alzheimer's disease temporal cortex is shown in FIG. 2(c). FIG. 2(d) is a dark-field photomicrograph of the same section as shown in FIG. 2(c), where .sup.125 I-.beta.-amyloid peptide.sup.1-40 revealed a more extensive distribution of plaques than did the anti-A4 antibody. FIG. 2(e) is a dark-field photomicrograph of thioflavin S staining in human Alzheimer's disease temporal cortex showing labelling of diffuse, compact and neuritic type plaques. FIG. 2(f) is a light-field photomicrograph of the same section as FIG. 2(e) bound with .sup.125 I-.beta.-amyloid peptide.sup.1-40, showing that all three types of plaques bind .sup.125 I-.beta.-amyloid peptide.sup.1-40. FIG. 2(g) is a dark-field photomicrograph of thioflavin S staining in human Alzheimer's disease temporal cortex showing labelling of a neuritic plaque and several adjacent neurons. FIG. 2(f) is a light-field photomicrograph showing the same section as FIG. 2(g), bound with .sup.125 I-.beta.-amyloid peptide.sup.1-40, showing that although both the core and halo of the plaque bind .sup.125 I-.beta.-amyloid peptide.sup.1-40, none of the labeled neurons show any .sup.125 I-.beta.-amyloid peptide binding.

Other Reference Publication (5):
J.E. Castano, et al., "In Vitro Formation of Amyloid Fibrils from Two Synthetic Peptides of Different Lengths Homologous to Alzheimer's Disease .beta.-Protein", Biochem. Biophys. Res. Commun., 141, 782-789 (1986).

Other Reference Publication (17):
D.A. Kirshner et al., "Synthetic peptide homologous to .beta. protien from Alzheimer disease forms amyloid-like fibrils in vitro", Proc. Natl. Acad. Sci. USA, 84, 6953-6957 (Oct. 1987).

CLAIMS:

(a) combining a sample of human tissue evidencing the presence of Alzheimer's amyloidosis, with an amount of labelled .beta.-amyloid peptide or labelled active peptide fragment thereof and an agent to be screened for a time effective to allow binding of the labelled .beta.-amyloid peptide of the labelled fragment thereof to amyloid plaques in the tissue;

(b) detecting the amount of labelled .beta.-amyloid peptide or labelled peptide fragment bound to the tissue sample; and

2. The method according to claim 1, wherein the label of the labelled .beta.-amyloid peptide or labelled active peptide fragment thereof comprises a radiolabel, an enzyme label, a fluorescent label, a chemiluminescent label, or an antigen label.

3. The method of claim 2, wherein the presence of the labelled .beta.-amyloid or labelled active peptide fragment thereof bound to amyloid plaques in the tissue sample is detected by autoradiography, positron emission tomography, nuclear magnetic resonance imaging, a gamma counter, or a scintillation counter.

6. The method according to claim 1, wherein the .beta.-amyloid peptide that is labelled has the amino acid sequence as set forth in SEQ. ID NO:1 prior to labelling.

7. The method according to claim 1, wherein the .beta.-amyloid peptide that is labelled has the amino acid sequence as set forth in SEQ. ID NO:2 prior to labelling.

L44: Entry 16 of 35 File: USPT

DOCUMENT-IDENTIFIER: US 5786137 A
TITLE: Method for assaying components in an enzyme-protein substrate system

YEAR ISSUED (ORACLE) (1):
1998

Detailed Description Text (13):
In measuring components involved in an enzyme-catalyzed reaction which leads to polymerization of the substrate (e.g., fibrinogen, collagen, C9, .beta.-amyloid protein, keratin, actin, tubulin, casein, and vimentin), the fluorescence of the label molecules of the polymerized substrate is quenched (reduced fluorescence emission), relative to the basal level of fluorescence prior to polymerization. The fluorescence quenching is due to enhanced neighbor-neighbor interactions between the fluorescent label molecules in the polymerized substrate. Measurement of quenching during the polymerization to an end point determination provides for information on the kinetics of the reaction. Measurement of fluorescence emission at the end point of quenching, relative to the basal level of fluorescence emission, can be used to quantitate enzyme activity, or enzyme activators or inhibitors (also relative to a reference level of fluorescence), depending on the components included in the reaction mixture.

Detailed Description Text (42):
FIG. 2A (30 mM FITC-fibrinogen polymerized in buffer 1) and in FIG. 2B (30 mM FITC-fibrinogen polymerized in buffer 2). At the lowest concentrations of thrombin used, a comparatively significant lag time was observed before dequenching proceeded. After generation of sufficient monomer and protofibril extension, lateral aggregation of fibrils proceeded. Once dequenching began, the maximum rate of dequenching with time appeared to be largely independent of thrombin concentration, as seen by the similar maximum slopes on curves in FIGS. 2A or 2B. Also, the final extent of quenching appeared to be modulated by the thickness of the fiber. At low thrombin concentration and low salt (buffer 1) the thicker fibers that formed (taking longer to form) resulted in a greater final extent of quenching compared to the thinner fibers formed at higher thrombin concentrations (FIG. 2A). In high salt, favoring the formation of thinner fibers, the modulation of the final extent of quenching by adjusting the thrombin concentration was qualitatively similar to reactions with low salt, but the final differences were much smaller. As seen in FIGS. 2A and 2B, at all thrombin concentrations, the lag phase was considerably longer in buffer 2 as compared to in buffer 1, suggesting the importance of lateral aggregation during dequenching (i.e., protofibril extension is fast at higher salt concentrations). However, when quenching achieved its maximal rate at a given stage of the polymerization, there was little difference between that rate for low (buffer 1) and high (buffer 2) salt concentrations.

Detailed Description Text (43):
Addition of unlabeled fibrinogen to the reaction mixture attenuated the thrombin-induced quenching in a dose-dependent manner (FIGS. 3A and 3B). This result would not be seen for a scattering-based mechanism of loss of fluorescence signal. Increasing the concentration of unlabeled fibrinogen reduces the probability of two FITC-fibrin monomers either being incorporated sequentially in a protofibril or interacting within a given cross-section of fiber. Increasing the unlabeled fibrinogen in the reaction altered the initial quenching rate slightly (at constant

thrombin concentration) and reduced the final extent of quenching in a dose-dependent manner. At a 5:1 ratio of unlabeled fibrinogen to FITC-fibrinogen, the quenching during polymerization was completely eliminated in fine fibers (FIG. 3A) while a small amount of quenching occurred at this ratio during polymerization of coarse fibers (FIG. 3B). This is consistent with an increased probability of two or more labeled FITC-fibrin monomers quenching each other in a thick fiber cross-section as compared to the quenching probability in a thin fiber-cross section which contains less fibrils. Thus, the quenching during polymerization is due to a mechanism involving molecular proximity which is achieved by two processes: fibril extension and fibril aggregation.

Detailed Description Text (80):
To illustrate plasmin-mediated fibrinolysis, plasmin (5 nM) was mixed for about 5 seconds in the reaction buffer containing between 0.5 to 30 nM FITC-.fibrin. Polymerization was monitored by exciting the reaction mixture with blue light (488 nm) and monitoring the fluorescence emission at 515 nm using a fluorimeter. The action of plasmin on FITC-fibrinogen produced a large increase in fluorescence intensity (dequenching) in a plasmin-dependent manner. During fibrinolysis, the monomers in the fibrils are degraded with subsequent loss of fibril/fiber structure. The early dequenching during fibrinolysis is due to the loss of quenching in the fibril/fiber due to loss of structure, and the generation of dequenched degradation products. The initial rate of dequenching was very similar for lysis of coarse and fine fibers (FIG. 9) and was dependent on the concentration of fibrin used at constant plasmin concentration. It was noted that the final dequenched state of lysed fibrin (non-crosslinked) achieved a final fluorescence emission identical to the final dequenched state of lysed fibrinogen. Also, the initial rate of fluorescence change for FITC-fibrin in fibrinolysis appeared similar to that seen for FITC-fibrinogen in fibrinogenolysis.

WEST

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DATE: Monday, July 22, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L44	L43 and fibril	35	L44
L43	(amyloid with label$) and @py<2000	103	L43
L42	l40 and @py<2000	23	L42
L41	l40 and (1990-1999).py.	0	L41
L40	prion with label$	40	L40
L39	(amyloid or fibril or prion) with label$	238	L39
L38	l33 same aggreg$	1	L38
L37	L33 with aggreg$	0	L37
L36	L33 and (amyloid or fibril or prion)	3	L36
L35	L33 same (amyloid or fibril or prion)	0	L35
L34	L33 with (amyloid or fibril or prion)	0	L34

L33	reactive cysteine	76	L33
L32	l22 same aggre$	0	L32
L31	l22 with aggre$	0	L31
L30	l22 with fibril	0	L30
L29	l22 with fibril	0	L29
L28	L22 and amyloid	2	L28
L27	L22 same amyloid	0	L27
L26	l22 with amyloid	0	L26
L25	l22 and prion	0	L25
L24	l22 with prion	0	L24
L23	L22 same prion	0	L23
L22	L21 or l20 or l19 or l18	167	L22
L21	labeled cysteine	48	L21
L20	cysteine labeled	70	L20
L19	cysteine labelled	41	L19
L18	(labelled cysteine)	24	L18
L17	acrylodan	8	L17
L16	prion and acrylodan	0	L16
L15	prion same acrylodan	0	L15
L14	prion with acrylodan	0	L14
L13	nanogold	9	L13
L12	prion same nanogold	0	L12
L11	prion with nanogold	0	L11
L10	prion with (side chain)	4	L10
L9	prion with mutagen$	4	L9
L8	prion with substit$	5	L8
L7	prion with cysteine	3	L7
L6	prion with substit$ with (resid$ or amino)	1	L6
L5	prion with substit with (resid$ or amino)	0	L5
L4	(prion and substit$).clm	0	L4
L3	(prion and (amino acid) and substitution).clm	0	L3
L2	(cysteine and prion).clm	0	L2

DB=DWPI,USPT,EPAB,JPAB,TDBD; PLUR=YES; OP=ADJ

L1	(prion and lysine).clm	0	L1

END OF SEARCH HISTORY